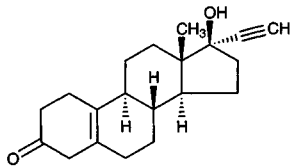
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 240.5

CHROMATOGRAM
Retention time: 24.038

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Norethynodrel



Molecular formula: $C_{20}H_{26}O_2$
Molecular weight: 298.43
CAS Registry No.: 68-23-5
Merck Index: 6791
Lednicer No.: 1 168

SAMPLE
Matrix: solutions

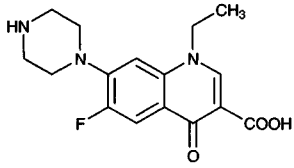
HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:THF:water 10:20:70
Flow rate: 2
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 6.2 (norethynodrel acetate)

OTHER SUBSTANCES
Simultaneous: ethinyl estradiol, norethindrone, norethindrone acetate, norgestrel

REFERENCE
Supelco Catalog, **1994**, p. 779.

Norfloxacin



Molecular formula: $C_{16}H_{18}FN_3O_3$
Molecular weight: 319.34
CAS Registry No.: 70458-96-7, 100587-52-8 (norfloxacin succinil)
Merck Index: 6793
Lednicer No.: 4 141, 143

SAMPLE
Matrix: aqueous humor, blood, tissue

Sample preparation: Homogenize sample in 1 M HCl, add MeCN, centrifuge, add an equal volume of dichloromethane, centrifuge, inject a 50-100 μL aliquot of the aqueous supernatant.

HPLC VARIABLES
Guard column: Corasil C18
Column: μBondapak C18
Mobile phase: MeCN:25 mM phosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide 9.5:90.5
Flow rate: 1.5
Injection volume: 50-100
Detector: F ex 272 em 370 (cut-off)

CHROMATOGRAM
Retention time: 6.5
Limit of detection: 0.1 ng/g

KEY WORDS
serum; rabbit; human; cornea

REFERENCE
Bron,A.M.; Péchinot,A.; Garcher,C.; Guyonnet,G.; Kazmierczak,A. Ocular penetration of topically applied norfloxacin 0.3% in the rabbits and in humans, *J.Ocul.Pharmacol.*, **1992**, *8*, 241–246.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 50 μL 400 μg/mL IS in water, vortex for 30 s. Add 500 μL MeCN, vortex for 1 min. Centrifuge at 6000 rpm for 10 min. Evaporate the supernatant to 200 μL at 40° under a stream of nitrogen, vortex for 30 s. Inject a 30-80 μL aliquot.

HPLC VARIABLES
Column: 100 × 8.0 4 μm Radial-pak Novapak C18
Mobile phase: MeCN:buffer 14:86 (Buffer was 2 g citric acid, 2 g sodium acetate, and 1 mL triethylamine in 1 L water.)
Flow rate: 2.5
Injection volume: 30-80
Detector: F ex 330 em 440

CHROMATOGRAM
Retention time: 4.0
Internal standard: acebutolol (7.4)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: pefloxacin
Simultaneous: ciprofloxacin, lomefloxacin, ofloxacin

KEY WORDS
serum; pharmacokinetics

REFERENCE
Abanmi,N.; Zaghloud,I.; El Sayed,N.; al-Khamis,K.I. Determination of pefloxacin and its main active metabolite in human serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1996**, *18*, 158–163.

SAMPLE
Matrix: blood
Sample preparation: Add 20 μL MeOH:0.1% trifluoroacetic acid 15:85 and 5 μL (sic) MeCN to 300 μL plasma. Centrifuge at 600 g for 10 min. Evaporate the supernatant under nitrogen at 40° for 30 min. Reconstitute the residue in 200 μL MeOH:0.1% trifluoroacetic acid 15:85. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 12.5 × 4 Zorbax RX-C18
Column: 150 × 4.6 5 μm Zorbax SB-C8
Mobile phase: MeCN:water:trifluoracetic acid 19:81:0.02
Flow rate: 1
Injection volume: 50
Detector: UV 279

CHROMATOGRAM
Retention time: 3.8-3.9
Internal standard: norfloxacin

OTHER SUBSTANCES
Extracted: ciprofloxacin, enrofloxacin

KEY WORDS
cat; plasma; norfloxacin is IS

REFERENCE
Kordick,D.L.; Papich,M.G.; Breitschwerdt,E.B. Efficacy of enrofloxacin or doxycycline for treatment of Bartonella henselae or Bartonella clarridgeiae infection in cats, *Antimicrob.Agents Chemother.*, **1997**, *41*, 2448–2455.

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 11:89 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1.2
Detector: UV 272

CHROMATOGRAM
Retention time: 8.07
Internal standard: pipemidic acid (4.14)

OTHER SUBSTANCES
Simultaneous: pefloxacin

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma + 1 mL 100 mM pH 7.0 K_2HPO_4 adjusted to pH 7.0 with 85% orthophosphoric acid + 100 μL 300 μg/mL nalidixic acid in water + 3 mL dichloromethane: isoamyl alcohol 9:1, shake vigorously for 10 min, centrifuge at 2270 g for 10 min. Remove 2 mL of the organic phase and evaporate it to dryness under a stream of nitrogen at 40°. Reconstitute residue in 100 μL MeOH:50 mM NaOH 2:1, vortex, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Chemcosorb 5-ODS-H

Mobile phase: MeOH:5 mM sodium lauryl sulfate 2:1, adjusted to pH 2.5 with 85% phosphoric acid (Better separation obtained at pH 2.35, J.Chromatogr. 1990, 530, 186.)
Column temperature: 40
Flow rate: 0.6
Injection volume: 10
Detector: UV 300

CHROMATOGRAM
Retention time: 6.5
Internal standard: nalidixic acid (5.0)

OTHER SUBSTANCES
Extracted: fenbufen, felbinac
Interfering: ofloxacin, enoxacin

KEY WORDS
plasma; rat

REFERENCE
Katagiri,Y.; Naora,K.; Ichikawa,N.; Hayashibara,M.; Iwamoto,K. Simultaneous determination of ofloxacin, fenbufen and felbinac in rat plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *431*, 135–142.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 250 μL 10% trichloroacetic acid, vortex for 10 s, centrifuge at >700 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 μBondapak C18 Radial-PAK
Mobile phase: MeOH:18 mM KH_2PO_4 containing 0.13 mM heptanesulfonic acid:concentrated phosphoric acid 30:70:0.1
Injection volume: 20
Detector: F ex 278 em 475

CHROMATOGRAM
Retention time: 5.6

KEY WORDS
serum

REFERENCE
Griggs,D.J.; Wise,R. A simple isocratic high-pressure liquid chromatographic assay of quinolones in serum, *J.Antimicrob.Chemother.*, **1989**, *24*, 437–445.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL pH 7.4 phosphate buffer + 50 μL 10 μg/mL β-hydroxypropyltheophylline in pH 7.4 phosphate buffer + 5 mL chloroform:isopropanol 80:20, shake on a rotary mixer for 15 min, centrifuge at 800 g for 5 min. Evaporate organic layer under nitrogen at 45°, sonicate residue with 100 μL mobile phase, inject 25 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 4.9 Spherisorb ODS
Column: 250 × 4.9 Sherisorb S5 ODS2
Mobile phase: MeCN:buffer 15:85 adjusted to pH 3.0 with 85% phosphoric acid immediately before use (Buffer was 4.54 g KH_2PO_4 + 5.94 g $Na_2HPO_4.2H_2O$ + 1.49 g tetrabutylammonium hydrogen sulfate per L.)
Flow rate: 1.3
Injection volume: 25
Detector: UV 280

CHROMATOGRAM
Retention time: 5.5
Internal standard: β-hydroxypropyltheophylline
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: theophylline, enoxacin, ciprofloxacin

KEY WORDS
plasma; rat

REFERENCE
Davis,J.D.; Aarons,L.; Houston,J.B. Simultaneous assay of fluoroquinolones and theophylline in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *621*, 105–109.

SAMPLE
Matrix: blood
Sample preparation: 150 μL Plasma + 10 μL 100 μg/mL enoxacin in water + 75 μL 10% trichloroacetic acid + 600 μL chloroform, vortex for 5 min, centrifuge at 13800 g for 10 min. Remove 500 μL of the organic layer and evaporate it to dryness under reduced pressure, reconstitute the residue in 150 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 80 × 4.6 5 μm Zorbax C8
Mobile phase: MeOH:0.01% trifluoroacetic acid 25:75
Flow rate: 1.2
Injection volume: 50
Detector: F ex 280 em 418

CHROMATOGRAM
Retention time: 6.9
Internal standard: enoxacin (5.8)
Limit of quantitation: 25 ng/mL

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Hussain,M.S.; Chukwumaeze-Obiajunwa,V.; Micetich,R.G. Sensitive high-performance liquid chromatographic assay for norfloxacin utilizing fluorescence detection, *J.Chromatogr.B*, **1995**, *663*, 379–384.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 1 mL 2.6 μg/mL N-ethylnorfloxacin in chloroform, vortex, centrifuge at 11000-12000 g for 1 min. Remove the organic layer and evaporate it to dryness under a stream of air at 60°, reconstitute the residue in 200 μL mobile phase, inject a 25-50 μL aliquot.

HPLC VARIABLES
Column: 40 × 3.2 3 μm RP-18 Spheri-3
Mobile phase: MeCN:10 mM pH 2.5 NaH_2PO_4 containing 1 mM triethylamine 11:89
Column temperature: 30
Flow rate: 1
Injection volume: 25-50
Detector: UV 279

CHROMATOGRAM
Retention time: 1.9
Internal standard: N-ethylnorfloxacin (Heat 3.2 g norfloxacin, 1.5 g triethylamine, and 12-20 mmoles ethyl iodide in 40 mL DMF at 80-90° with stirring for 2 h, concentrate to dryness,

recrystallize from chloroform/benzene to give N-ethylnorfloxacin (mp 251-3°) (J.Med.Chem. 1980, 23, 1358)) (2.9)
Limit of detection: 20 ng/mL

KEY WORDS
serum

REFERENCE
Wallis,S.C.; Charles,B.G.; Gahan,L.R. Rapid and economical high-performance liquid chromatographic method for the determination of norfloxacin in serum using a microparticulate C18 guard cartridge, *J.Chromatogr.B*, **1995**, *674*, 306–309.

SAMPLE
Matrix: blood, dialysate
Sample preparation: 100 μL Plasma or dialysate + 400 μL MeOH, vortex, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: strong cation exchange
Mobile phase: MeCN:100 mM pH 3 citrate buffer 20:80
Detector: F ex 278 em 440

CHROMATOGRAM
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: pefloxacin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Rose,T.F.; Bremner,D.A.; Collins,J.; Ellis-Pegler,R.; Isaacs,R.; Richardson,R.; Small,M. Plasma and dialysate levels of pefloxacin and its metabolites in CAPD patients with peritonitis, *J.Antimicrob.Chemother.*, **1990**, *25*, 657–664.

SAMPLE
Matrix: blood, formulations
Sample preparation: Blood. Centrifuge 200 μL fresh blood at 3000 rpm for 10 min. Inject an aliquot of the plasma. Formulations. Completely dissolve 50 mg sample in 20 mL MeOH, sonicate. Filter insoluble material and adjust filtrate to 50 mL with MeOH. Inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 Cosmosil 5C18 (Nacalai Tesque, Japan)
Mobile phase: MeCN:solution 1:6.5 (Solution was 1 g sodium acetate trihydrate, 2 g citric acid monohydrate and 1 mL triethylamine in 1 L water (?).)
Column temperature: 40
Flow rate: 1.5
Injection volume: 100
Detector: UV 277

CHROMATOGRAM
Internal standard: p-nitrophenylacetic acid

KEY WORDS
freeze-dried formulations; plasma; rat; egg albumin; olive oil

REFERENCE
Tsuji,Y.; Kakegawa,H.; Miyataka,H.; Nishiki,M.; Matsumoto,H.; Satoh,T. Pharmaceutical properties of freeze-dried formulations of egg albumin, several drugs and olive oil, *Biol.Pharm.Bull.*, **1996**, *19*, 636–640.

SAMPLE

Matrix: blood, tissue

Sample preparation: Plasma. Mix 500 μL plasma with 5 μg IS, add 4 mL dichloromethane and 100 μL pH 7.4 phosphate buffer, agitate for 10 min, centrifuge at 5300 g for 10 min. Collect 3.5 mL organic phase. Add 4 mL dichloromethane to the aqueous phase again, agitate, centrifuge. Combine the organic phases, evaporate at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot. Tissue. Mix 500 μL epiploic-fat and 4 mL dichloromethane and keep at 4°, add 5 μg IS, mix by using an automatic grinder (Ultra Turrax, Ika-Werk, Stauffen, Germany). Collect the mixture, centrifuge at 5300 g for 10 min. Add 4 mL 100 mM NaOH to the dichloromethane, agitate for 10 min, centrifuge at 5300 g for 5 min. Eliminate the organic phase, adjust the aqueous phase to pH 7.4 with concentrated trichloroacetic acid, add 4 mL dichloromethane, agitate for 10 min and centrifuge at 5300 g for 5 min. Evaporate the organic phase at 60°. Reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 25 × 4 5 μm 100 RP-18 Lichrosphere
Column: 125 × 4 5 μm 100 RP-18 endcapped Lichrosphere
Mobile phase: MeCN:pH 4.8 citrate buffer 85:15
Flow rate: 1
Injection volume: 20
Detector: F ex 330 em 418

CHROMATOGRAM

Internal standard: 4844P (pefloxacin analog)
Limit of quantitation: 25 ng/mL

OTHER SUBSTANCES

Extracted: pefloxacin

KEY WORDS

epiploic-fat; plasma; pharmacokinetics; fat

REFERENCE

Jacoberger,B.; Ubeaud,G.; Freys,G.; Pottecher,T.; Jung,L.; Koffel,J.C. Concentrations of pefloxacin in plasma and tissue after administration as surgical prophylaxis, *Antimicrob.Agents Chemother.*, **1998**, *42*, 425–427.

SAMPLE

Matrix: blood, tissue

Sample preparation: Tissue. Homogenize 100-250 mg tissue with 5 mL 500 mM pH 7.0 phosphate buffer, remove a 1 mL aliquot, add 100 μL 10 μg/mL IS, mix, add 10 mL chloroform: isopentanol 90:10, shake for 15 min, centrifuge , repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of air at 60°, reconstitute the residue in 100 μL 1% ammonia, inject a 25 μL aliquot. Plasma. 250-500 μL Plasma + 100 μL 10 μg/mL IS + 1 mL 500 mM pH 7.0 sodium phosphate buffer, mix, add 10 mL chloroform:isopentanol 90:10, shake for 15 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of air at 60°, reconstitute the residue in 100 μL 1% ammonia, inject a 25 μL aliquot.

HPLC VARIABLES

Column: 100 × 5 10 μm Nucleosil C18
Mobile phase: MeCN:water 15:85 containing 2 g/L sodium acetate trihydrate, 2 g/L citric acid monohydrate, and 1 mL/L triethylamine
Flow rate: 2
Injection volume: 25
Detector: F ex 330 em 440

CHROMATOGRAM

Retention time: 2.8
Internal standard: 1-allyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)quinoline-3-carboxylic acid (Roger Bellon Laboratories) (6.6)
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, pefloxacin

KEY WORDS
plasma; prostate

REFERENCE
Montay,G.; Tassel,J.P. Improved high-performance liquid chromatographic determination of pefloxacin and its metabolite norfloxacin in human plasma and tissue, *J.Chromatogr.*, **1985**, *339*, 214–218.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Serum, plasma. Dilute serum or plasma 1:2 to 1:10 with 30 mM phosphoric acid, centrifuge, inject a 20 μL aliquot of supernatant. Urine. Dilute urine 1:10 to 1:100 with 30 mM phosphoric acid, centrifuge, inject a 20 μL aliquot of supernatant. Tissue (lung, gut). Cut tissue with a scalpel, homogenize with 1-3 mL buffer, centrifuge at 9600 g for 5 min three times, inject a 20 μL aliquot. Tissue (chondral). Cut tissue with a scalpel, homogenize with 3-6 mL buffer in an ice bath for 2-3 min, centrifuge at 9600 g for 5 min four or five times, inject a 100 μL aliquot. Dilute human pleural samples with buffer, centrifuge, inject a 20 μL aliquot. (Buffer was 66.6 mM K_2HPO_4 adjusted to pH 7.40 with KH_2PO_4.)

HPLC VARIABLES
Column: 200 × 4 5 μm Nucleosil C18
Mobile phase: MeOH:MeCN:buffer 13:7:80, adjusted to pH 3.0 with phosphoric acid (Buffer was 15 mM phosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide.)
Flow rate: 1
Injection volume: 20-100
Detector: F ex 278 em 446

CHROMATOGRAM
Retention time: 4
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES
Simultaneous: ofloxacin, ciprofloxacin

KEY WORDS
serum; plasma; lung; gut; pleural; chondral

REFERENCE
Knöller,J.; König,W.; Schönfeld,W.; Bremm,K.D.; Köller,M. Application of high-performance liquid chromatography of some antibiotics in clinical microbiology, *J.Chromatogr.*, **1988**, *427*, 257–267.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 500 μL MeCN to 500 μL plasma or urine diluted with water, vortex vigorously for 20 s, centrifuge at 4000 rpm for 2 min, mix a 250 μL aliquot of the supernatant with 500 μL 100 mM perchloric acid containing 20 mM triethylamine, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 80 × 4.0 3 μm Nucleosil 3C18
Mobile phase: MeOH:100 mM perchloric acid containing 20 mM triethylamine 30:70
Column temperature: 40
Flow rate: 1
Injection volume: 20
Detector: F ex 300 em 450

CHROMATOGRAM
Retention time: 2.09

OTHER SUBSTANCES
Noninterfering: acyclovir, ampicillin, amoxicillin, clavulanic acid, doxycycline, erythromycin, lansoprazole, metronidazole, minocycline, omeprazole, penicillin V, trimethoprim

KEY WORDS
plasma

REFERENCE
Mascher,H.J.; Kikuta,C. Determination of norfloxacin in human plasma and urine by high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.A*, **1998**, *812*, 381–385.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Wash a C18 Sep-Pak cartridge with 1 mL 4% methanolic phosphoric acid and 10 mL water. Add 1 mL plasma to the cartridge, wash with 6 mL water, elute with 1 mL 4% methanolic phosphoric acid then with 1 mL water. Combine the eluates, make up to 2 mL with water, inject a 50 μL aliquot. Urine. Dilute 100 μL urine with 900 μL mobile phase and directly inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: μBondapak C18-Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:MeCN:water 300:50:700 + 1.74 g K_2HPO_4 + 20 mg sodium heptanesulfonate, pH adjusted to 3 with phosphoric acid
Flow rate: 2
Injection volume: 10 (urine), 50 (plasma)
Detector: F ex 285 em 440

CHROMATOGRAM
Retention time: 4
Limit of detection: 500 ng/mL (urine), 20 ng/mL (plasma)

KEY WORDS
plasma; SPE

REFERENCE
Gutzler,F.; de Vries,J.X. Bestimmung von Norfloxacin in Plasma und Urin durch Hochdruckflüssigkeitschromatographie, *Fortschr.Antimikr.Antineoplast.Chemother.*, **1984**, *3*, 673–677.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute with one or more volumes of water, filter (0.6 μm)

HPLC VARIABLES
Column: 200 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:25 mM orthophosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide 11:89
Flow rate: 1.5
Injection volume: 10-20
Detector: F ex 278 em 445

CHROMATOGRAM
Retention time: 2.8
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Noninterfering: trimethoprim, sulfamethoxazole, netilmicin, metronidazole, penicillin G, cloxacillin, doxycycline, cefuroxime, erythromycin, salicylic acid, digoxin, furosemide, acetaminophen, prednisolone, warfarin, dextropropoxyphene
Interfering: ciprofloxacin

KEY WORDS
serum

REFERENCE
Nilsson-Ehle,I. Assay of ciprofloxacin and norfloxacin in serum and urine by high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *416*, 207–211.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 250 μL Plasma + 250 μL 10 μg/mL IS in water + 750 μL MeOH, stir, centrifuge at 2000 rpm for 5 min, inject a 50 μL aliquot of the supernatant. Urine. 500 μL Urine + 3.5 mL 8 μg/mL IS in water, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 Nucleosil C8
Mobile phase: MeCN:water:triethylamine:formic acid 11:87.5:0.1:1 containing 0.2% sodium acetate and 0.1% formic acid
Flow rate: 1
Injection volume: 50
Detector: F ex 280 em 450

CHROMATOGRAM
Internal standard: 1-ethyl-6-chloro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (RP 41983)
Limit of quantitation: 500 ng/mL (urine), 100 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: pefloxacin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Humbert,G.; Brumpt,I.; Montay,G.; Le Liboux,A.; Frydman,A.; Borsa-Lebas,F.; Moore,N. Influence of rifampin on the pharmacokinetics of pefloxacin, *Clin.Pharmacol.Ther.*, **1991**, *50*, 682–687.

SAMPLE
Matrix: cells
Sample preparation: Incubate cells in 2 mL 100 mM pH 3.0 glycine-HCl buffer for 2 h at room temperature, centrifuge at 5600 g for 5 min, inject an aliquot.

HPLC VARIABLES
Column: Bondapak C18
Mobile phase: MeCN:25 mM phosphoric acid adjusted to pH 3.0 with tetrabutylammonium hydroxide 25:75
Flow rate: 1.5
Detector: F ex 340 em 425

OTHER SUBSTANCES
Also analyzed: ciprofloxacin, fleroxacin, lomefloxacin, ofloxacin, temafloxacin

REFERENCE
Pascual,A.; Garcia,I.; Conejo,M.C.; Perea,E.J. Fluorometric and high-performance liquid chromatographic measurement of quinolone uptake by human neutrophils, *Eur.J.Clin.Microbiol.Infect.Dis.*, **1991**, *10*, 969–971.

SAMPLE
Matrix: hair
Sample preparation: Wash hair successively with 0.1% sodium dodecyl sulfate and water for 30 min, repeat twice, blot between 2 sheets of paper towel, allow to dry at room temperature. Take a 1 cm fragment of hair, add 500 μL 1 M NaOH, heat at 80° for 30 min, cool, add 500

μL 1 M HCl, add 1 mL 100 mM pH 4.6 potassium hydrogen citrate buffer, add 50 μL 1 μg/mL IS in water. Add the mixture to a Bond-Elut C8 cartridge, elute with 2 mL THF:25 mM orthophosphoric acid 20:80, evaporate eluate to dryness in vacuum, dissolve residue in 150 μL mobile phase, vortex, inject a 60 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Tosoh 5 μm TSKgel ODS-80Ts
Mobile phase: MeCN:25 mM orthophosphoric acid adjusted to pH 3.0 with 0.5 M tetra-n-butylamine hydroxide 5:95
Column temperature: 40
Flow rate: 1
Injection volume: 60
Detector: F ex 280 em 445

CHROMATOGRAM
Retention time: 11.7
Internal standard: (R)-9-fluoro-2,3-dihydro-3-methyl-10-(4-ethyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid (DS-4632) (10.2)
Limit of detection: 0.2 ng/mL

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, ofloxacin (determine at F ex 295 em 490)

KEY WORDS
SPE

REFERENCE
Mizuno,A.; Uematsu,T.; Nakashima,M. Simultaneous determination of ofloxacin, norfloxacin and ciprofloxacin in human hair by high-performance liquid chromatography and fluorescence detection, *J.Chromatogr.B*, **1994**, *653*, 187–193.

SAMPLE
Matrix: perfusate

HPLC VARIABLES
Column: 250 × 4.6 Spheris C18 (Phase Separations)
Mobile phase: MeCN:15 mM tetrabutylammonium iodide 5:95
Flow rate: 1
Detector: UV 275

CHROMATOGRAM
Internal standard: pipemidic acid

REFERENCE
Lin,H.-H.; Hsu,L.-R.; Wu,P.-C.; Tsai,Y.-H. Increased norfloxacin skin permeability for fatty alcohol propylene glycol (FAPG) ointment by optimized process of preparation: Behavior of stearic acid in stratum corneum lipids, *Biol.Pharm.Bull.*, **1995**, *18*, 1560–1565.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 450 μg/mL solution in MeCN:water 50:50. 5 mL Solution + 5 mL THF + 200 molar excess of acetic anhydride + 3 molar excess of 1 M NaOH, sonicate for 15 min, add 15 mL mobile phase, sonicate for 15 min, cool to room temperature, make up to 50 mL with mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:buffer 35:65 (Buffer was prepared by mixing equal volumes of 20 mM citric acid and 20 mM sodium citrate, pH adjusted to 2.4 with perchloric acid.)
Flow rate: 1
Injection volume: 50

Detector: UV 280

CHROMATOGRAM
Retention time: 5.9

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, sarafloxacin, temafloxacin

KEY WORDS
derivatization

REFERENCE
Morley,J.A.; Elrod,L.,Jr. Determination of fluoroquinolone antibacterials as N-Acyl derivatives, *Chromatographia*, **1993**, *37*, 295–299.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 20 μg/mL solution in MeCN:water 10:90, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:25 mM phosphoric acid 7:93, adjusted to pH 3.09 with 100 mM tetrabutylammonium hydroxide
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 8.6

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, ofloxacin (UV 295), pipemidic acid

REFERENCE
Barbosa,J.; Bergés,R.; Sanz-Nebot,V. Linear solvation energy relationships in reversed-phase liquid chromatography. Prediction of retention of several quinolones, *J.Liq.Chromatogr.*, **1995**, *18*, 3445–3463.

SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 μm) a solution in MeCN:water 10:90, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 RP-18
Mobile phase: MeCN:buffer 7:93 (Buffer was 25 mM phosphoric acid adjusted to pH 3.89 with 100 mM tetrabutylammonium hydroxide.)
Flow rate: 1
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 8.8

OTHER SUBSTANCES
Simultaneous: ciprofloxacin, enoxacin, fleroxacin, ofloxacin (UV 295), pipemidic acid

REFERENCE
Barbosa,J.; Bergés,R.; Sanz-Nebot,V. Solvatochromic parameter values and pH in aqueous-organic mixtures used in liquid chromatography. Prediction of retention of a series of quinolones, *J.Chromatogr.A*, **1996**, *719*, 27–36.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) eye tissue with 3 mL 50 mM pH 5.8 sodium phosphate-citrate buffer and IS, centrifuge. Add the supernatant to 7 mL chloroform, agitate, centrifuge at 1000 g for 10 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Nucleosil C8
Mobile phase: MeCN:water 26:74 containing 2 g/L sodium acetate trihydrate, 2 g/L citric acid monohydrate, 4 mL/L triethylamine, and 2 mL/L formic acid, pH 4.8
Flow rate: 1
Injection volume: 5-20
Detector: UV 280

CHROMATOGRAM
Retention time: 2.19
Internal standard: 1-allyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)quinoline-3-carboxylic acid (?) 4662 P (Roger-Bellon Laboratories) (2.95)
Limit of detection: 5 ng

OTHER SUBSTANCES
Extracted: pefloxacin

KEY WORDS
rabbit; eye; pharmacokinetics

REFERENCE
Cochereau-Massin,I.; Bauchet,J.; Faurisson,F.; Vallois,J.M.; Lacombe,P.; Pocidalo,J.J. Ocular kinetics of pefloxacin after intramuscular administration in albino and pigmented rabbits, *Antimicrob.Agents Chemother.*, **1991**, *35*, 1112–1115.

SAMPLE
Matrix: urine
Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:MeCN:100 mM pH 5.75 phosphate buffer 24.1:2.6:73.3
Flow rate: 1
Detector: F ex 275 em 415

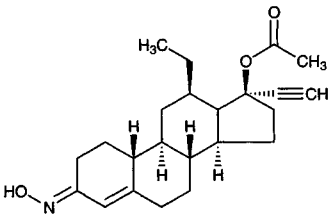
CHROMATOGRAM
Limit of quantitation: 3.13 μg/mL

OTHER SUBSTANCES
Extracted: pefloxacin

KEY WORDS
pharmacokinetics

REFERENCE
Jaehde,U.; Sörgel,F.; Stephan,U.; Schunack,W. Effect of an antacid containing magnesium and aluminum on absorption, metabolism, and mechanism of renal elimination of pefloxacin in humans, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1129–1133.

Norgestimate



Molecular formula: $C_{23}H_{31}NO_3$
Molecular weight: 369.50
CAS Registry No.: 35189-28-7
Merck Index: 6796

SAMPLE
Matrix: blood
Sample preparation: 1 mL Serum + 3 mL MTBE, vortex for 1 min, centrifuge at 1500 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 100 μL MeOH, inject an aliquot.

HPLC VARIABLES
Guard column: 18 mm long (Brownlee)
Column: 300 × 3.6 10 μm μBondapak C18
Mobile phase: MeOH:water 80:20
Flow rate: 1
Detector: UV 254 or RIA

CHROMATOGRAM
Retention time: 9.3, 9.8 (syn and anti)

OTHER SUBSTANCES
Extracted: metabolites, norgestrel

KEY WORDS
serum

REFERENCE
Wong,F.A.; Juzwin,S.J.; Tischio,N.S.; Flor,S.C. Determination of norgestimate in serum by automated high-performance liquid chromatography and subsequent radioimmunoassay, *J.Liq.Chromatogr.*, **1995**, *18*, 1851–1861.

SAMPLE
Matrix: culture media
Sample preparation: Extract culture medium twice with 2 volumes of ether, combine the extracts and evaporate them to dryness, reconstitute with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 Techopak 10 C18 (HPLC Technology)
Mobile phase: MeOH:water 70:30
Flow rate: 1.5
Detector: UV 240, radioactivity

CHROMATOGRAM
Retention time: 13.5, 15.5 (racemate)

OTHER SUBSTANCES
Extracted: metabolites, norgestrel

KEY WORDS
tritium labeled

REFERENCE
Wild,M.J.; Rudland,P.S.; Back,D.J. Metabolism of the oral contraceptive steroids ethynylestradiol and norgestimate by normal (Huma 7) and malignant (MCF-7 and ZR-75-1) human breast cells in culture, *J.Steroid Biochem.Mol.Biol.*, **1991**, *39*, 535–543.

SAMPLE
Matrix: formulations
Sample preparation: 5 Tablets + 2 glass beads + 25 mL 50 μg/mL dibutyl phthalate in MeOH, vortex 15 min or until tablets have completely disintegrated, sonicate 5 min, filter (2 μm), inject 25 μL aliquot.

HPLC VARIABLES
Column: 50 × 4.5 5μm IBM C18
Mobile phase: MeOH:THF:water 10:25:65
Flow rate: 2.1
Injection volume: 25
Detector: UV 230

CHROMATOGRAM
Retention time: 3.5
Internal standard: dibutyl phthalate

OTHER SUBSTANCES
Simultaneous: ethinylestradiol, degradation products

KEY WORDS
tablets; stability-indicating

REFERENCE
Lane,P.A.; Mayberry,D.O.; Young,R.W. Determination of norgestimate and ethinyl estradiol in tablets by high-performance liquid chromatography, *J.Pharm.Sci.*, **1987**, *76*, 44–47.

SAMPLE
Matrix: microsomal incubations, mucosal fluid
Sample preparation: Mucosal fluid. Extract 1 mL mucosal fluid twice with 5 mL diethyl ether, evaporate extracts to dryness, resuspend residue in 100 μL MeOH, inject an aliquot. Microsomal incubations. Extract 2.5 mL microsomal incubation with 5 mL diethyl ether, proceed as before.

HPLC VARIABLES
Guard column: on-line guard column
Column: 100 × 8 μBondapak radial compression module
Mobile phase: MeOH:water 70:30
Flow rate: 1.5
Injection volume: 100
Detector: UV 240

CHROMATOGRAM
Retention time: 17.5

OTHER SUBSTANCES
Simultaneous: 3-ketonorgestimate, 17-deacetylnorgestimate, norgestrel

REFERENCE
Madden,S.; Back,D.J. Metabolism of norgestimate by human gastrointestinal mucosa and liver microsomes in vitro, *J.Steroid Biochem.Mol.Biol.*, **1991**, *38*, 497–503.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 μBondapak C18
Mobile phase: Dioxane:water 50:50 (CAUTION! Dioxane is a carcinogen!)
Flow rate: 1.4
Detector: UV 254

OTHER SUBSTANCES
Simultaneous: levonorgestrel

REFERENCE
Killinger,J.; Hahn,D.W.; Phillips,A.; Hetyei,N.S.; McGuire,J.L. The affinity of norgestimate for uterine progestogen receptors and its direct action on the uterus, *Contraception*, **1985**, *32*, 311–319.

SAMPLE
Matrix: tissue
Sample preparation: Incubate endometrial tissue with buffer, remove tissue, extract medium twice with 2 volumes of diethyl ether, evaporate to dryness, reconstitute in a small volume of MeOH, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 Technopak 10 C18
Mobile phase: MeOH:water 70:30
Flow rate: 1.5
Detector: UV 240

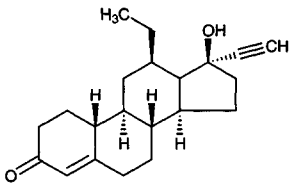
CHROMATOGRAM
Retention time: 15, 18 (syn and anti)

OTHER SUBSTANCES
Simultaneous: norgestrel, metabolites

KEY WORDS
endometrial tissue

REFERENCE
Wild,M.J.; Rudland,P.S.; Back,D.J. Metabolism of the oral contraceptive steroids ethynylestradiol, norgestimate and 3-ketodesogestrel by a human endometrial cancer cell line (HEC-1A) and endometrial tissue *in vitro*, *J.Steroid Biochem.Mol.Biol.*, **1993**, *45*, 407–420.

Norgestrel



Molecular formula: $C_{21}H_{28}O_2$
Molecular weight: 312.45
CAS Registry No.: 797-63-7, 797-64-8 ((-) form), 6533-00-2
Merck Index: 6797
Lednicer No.: 1 167, 2 151, 3 84

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 241.7

CHROMATOGRAM
Retention time: 21.565

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Centrifuge oil formulation at 30° at 2000 rpm for 30 min, filter (Whatman No. 1 paper), collect the last 4 mL of the filtrate. Dilute a 10 μL aliquot to 10 mL with MeCN:water 60:40 containing 0.3% Tween 80, add a 2 mL aliquot and add it to 1 mL 3.33 μg/mL progesterone, vortex for 10 s, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 Novapak C18
Mobile phase: MeCN:water 60:40
Flow rate: 2
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Internal standard: progesterone

KEY WORDS
oils

REFERENCE
Gao,Z.-H.; Shukla,A.J.; Johnson,J.R.; Crowley,W.R. Controlled release of a contraceptive steroid from biodegradable and injectable gel formulations: In vitro evaluation, *Pharm.Res.*, **1995**, *12*, 857–863.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:water 50:50
Column temperature: 37
Flow rate: 2.0
Detector: UV 243

CHROMATOGRAM
Retention time: 4.07

REFERENCE
Kim,D.-D.; Kim,J.L.; Chien,Y.W. Mutual hairless rat skin permeation-enhancing effect of ethanol/water system and oleic acid, *J.Pharm.Sci.*, **1996**, *85*, 1191–1195.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 μBondapak C18
Mobile phase: Dioxane:water 50:50 (CAUTION! Dioxane is a carcinogen!)
Flow rate: 1.4
Detector: UV 254

OTHER SUBSTANCES
Simultaneous: norgestimate

REFERENCE
Killinger,J.; Hahn,D.W.; Phillips,A.; Hetyei,N.S.; McGuire,J.L. The affinity of norgestimate for uterine progestogen receptors and its direct action on the uterus, *Contraception*, **1985**, *32*, 311–319.

SAMPLE
Matrix: solutions
Sample preparation: Direct injection

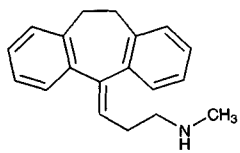
HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil C-18 ODS-3
Mobile phase: MeCN:water 50:50
Flow rate: 2
Detector: UV 243

CHROMATOGRAM
Retention time: 6.0

KEY WORDS
see also J.Pharm.Sci. 1989; 78; 477

REFERENCE
Catz,P.; Friend,D.R. In vitro evaluations of transdermal levonorgestrel, *Drug Des.Deliv.*, **1990**, *6*, 49–60.

Nortriptyline



Molecular formula: $C_{19}H_{21}N$
Molecular weight: 263.38
CAS Registry No.: 72-69-5, 894-71-3 (HCl)
Merck Index: 6812
Lednicer No.: 1 151

SAMPLE
Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Chop 5-g tissue and homogenize (Ultra Turrax T25) at 8500, 9500, 13500, 20500, and 24000 rpm for 1 min each. Add homogenate to 20 mL water. Dilute blood, urine, gastric contents, and bile four times with water. Mix 4 mL sample with 100 μL 400 μg/mL IS and 2 mL 500 mM NaOH, vortex briefly, add 4 mL heptane:isoamyl alcohol 98.5:1.5 and mix for 15 min (Spiramix 10, Denley, UK). Separate the organic layer, add 4 mL heptane:isoamyl alcohol 98.5:1.5 to extraction sample, mix. Combine the organic layers and extract them with 2 mL 50 mM sulfuric acid. Make the acid layer alkaline with 1 mL 1.0 M pH 9.0 carbonate/bicarbonate buffer and mix with 2 mL toluene:isoamyl alcohol 85:15 for 15 min. Evaporate the organic layer to dryness, reconstitute the residue in 100 μL MeOH and inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Apex II ODS

Column: 150 × 4.6 5 μm Apex II OD
Mobile phase: MeCN:pH 3 phosphate buffer:n-nonylamine 40-50:60:0.12
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3.70
Internal standard: doxepin (2.99)

OTHER SUBSTANCES
Extracted: amitriptyline

KEY WORDS
liver; lung; muscle; urine; pericardial fluid

REFERENCE
Pounder,D.J.; Adams,E.; Fuke,C.; Langford,A.M. Site to site variability of postmortem drug concentrations in liver and lung, *J.Forensic Sci.*, **1996**, *41*, 927–932.

SAMPLE
Matrix: blood
Sample preparation: Add 250 μL 2 M sodium carbonate to 500 μL plasma. Add 100 μL 1 μg/mL IS in MeOH, extract with 10 mL n-hexane. Shake for 30 min and centrifuge at 3000 g for 10 min. Cool in a dry ice-acetone bath. Add 200 μL 0.3% phosphoric acid to upper organic layer. Shake for 10 min and centrifuge at 3000 g for 10 min. Separate the organic layer. Inject a 100 μL aliquot of the acidic aqueous layer.

HPLC VARIABLES
Column: 250 × 4.6 5 μm C18 Symmetry (Waters Millipore, USA)
Mobile phase: MeCN:67 mM potassium phosphate buffer adjusted to pH 3.0 with phosphoric acid 35:65 (After each chromatographic session wash the column with 200 mL MeCN:water 50:50.)
Flow rate: 1.2
Injection volume: 100
Detector: UV 226, UV 254, UV 400

CHROMATOGRAM
Retention time: 10.45
Internal standard: clovoxamine (6.5)
Limit of quantitation: 5 ng/mL (UV 226, UV 400 nm); 7 ng/mL (UV 254)

OTHER SUBSTANCES
Extracted: metabolites, amitriptyline, clomipramine, desipramine, fluoxetine imipramine, maprotiline
Simultaneous: amineptine, carbamazepine, chlordiazepoxide, chlorpromazine, clonazepam, clorazepate, clozapine, cyamemazine, desmethylmaproptiline, desmethylvenlafaxine, doxepin, flunitrazepam, fluvoxamine, haloperidol, levomepromazine, lorazepam, loxapine, mianserine, sulpiride, trimipramine, venlafaxine, viloxazine, zolpidem, zopiclone
Noninterfering: diazepam, valproic acid

KEY WORDS
plasma

REFERENCE
Aymard,G.; Livi,P.; Pham,Y.T.; Diquet,B. Sensitive and rapid method for the simultaneous quantification of five antidepressants with their respective metabolites in plasma using high-performance liquid chromatography with diode-array detection, *J.Chromatogr.B*, **1997**, *700*, 183–189.

SAMPLE
Matrix: blood

Sample preparation: Condition a 1 mL 30 mg Oasis HLB SPE cartridge with 1 mL MeOH and 1 mL water. Acidify (?) mL serum with 20 μL phosphoric acid, vortex for 5 s, add to the SPE cartridge, wash with 1 mL MeOH :water 5:95, elute with 1 mL MeOH. Evaporate the eluate to dryness at 40° under a stream of nitrogen. Reconstitute the residue with 200 μL MeOH:20 mM pH 7 phosphate buffer 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 3.9 Sentry
Column: 150 × 3.9 5 μm Symmetry C18 (Waters)
Mobile phase: MeOH:20 mM pH 7 potassium phosphate 70:30
Column temperature: 35
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8
Internal standard: nordoxepin (4.9)

OTHER SUBSTANCES
Simultaneous: amitriptyline, doxepin

KEY WORDS
pig; serum; SPE

REFERENCE
Cheng,Y.-F.; Phillips,D.J.; Neue,U.; Bean,L. Solid-phase extraction for the determination of tricyclic antidepressants in serum using a novel polymeric extraction sorbent, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2461–2473.

SAMPLE
Matrix: blood, microsomal incubations
Sample preparation: Vortex 1 mL plasma or microsomal incubation with 200 μL 1 μg/mL desipramine and 100 μL 5 M NaOH for 10 s, add 5 mL butan-1-ol:hexane 2:98, vortex for 1 min, centrifuge at 2000 g and 4° for 5 min, evaporate the organic phase to dryness at 40° using a vacuum vortex evaporator, reconstitute the residue in 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 5 μm Nova-Pak C18
Mobile phase: MeCN:buffer 30:70 (Buffer was water containing 1% triethylamine, adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 2
Injection volume: 50
Detector: UV 240

CHROMATOGRAM
Retention time: 8.5
Internal standard: desipramine (6.3)
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: amitriptyline
Noninterfering: diazepam, furafylline, hydroxyamitriptyline, hydroxynortriptyline, quinidine, mephenytoin, triacetyloleandomycin
Interfering: ketoconazole

KEY WORDS
human; liver; rat; plasma

REFERENCE
Ghahramani,P.; Lennard,M.S. Quantitative analysis of amitriptyline and nortriptyline in human plasma and liver microsomal preparations by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *685*, 307–313.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 7.0 (A), 9.2 (B)
Internal standard: diazepam (4.4, A)
Limit of quantitation: 50 ng/mL(serum, urine), 500 ng/mL (tissue)

OTHER SUBSTANCES
Extracted: amitriptyline, amoxapine, clomipramine, desipramine, dothiepin, doxepin, imipramine, maprotiline, melitracen, mianserin
Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

KEY WORDS
serum; brain; liver

REFERENCE
Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 500 μL 3 M ammonia solution and 7 mL n-pentane:isopropanol 95:5 to 2 mL plasma or urine, shake in an overhead shaker for 20 min, let stand for 10 min. Transfer the upper organic layer to a tube containing 1 mL 100 mM HCl, shake for 20 min, let stand for 5 min. Aspirate the organic phase to waste, wash the remaining aqueous layer with 3 mL pentane by shaking for 10 min. Add 500 μL 3 M ammonia solution and 6 mL a-pentane: isopropanol 95:5 to the washed aqueous layer, shake for 20 min, evaporate the organic layer under a stream of nitrogen at 65°, reconstitute the residue with 160 μL mobile phase, inject 60 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.5 3 μm Spherisorb silica
Mobile phase: MeOH:hexane:nonylamine 5:95:0.3
Flow rate: 1
Injection volume: 60
Detector: UV 254

CHROMATOGRAM
Retention time: 10
Internal standard: nortriptyline

OTHER SUBSTANCES
Extracted: N-desmethyldoxepin, doxepin

KEY WORDS
dog; human; plasma; normal phase; nortriptyline is IS

REFERENCE
Yan,J.; Hubbard,J.W.; McKay,G.; Midha,K.K. Stereoselective and simultaneous measurement of cis- and trans-isomers of doxepin and N-desmethyldoxepin in plasma or urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *691*, 131–138.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 15.603

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: serum
Sample preparation: 1 mL Serum + 500 μL 750 mM pH 10 sodium bicarbonate/carbonate buffer + 50 μL IS in EtOH:water 50:50 + 8 mL heptane:isoamyl alcohol 98:2, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min, freeze in dry ice/EtOH. Remove the organic layer and add it to 150 μL 22 mM pH 2.5 KH_2PO_4/phosphoric acid buffer, shake at 250 cycles/min for 5 min, centrifuge at 1500 g for 10 min, freeze in dry ice/EtOH. Discard the organic layer, inject a 65 μL aliquot of the aqueous layer.

HPLC VARIABLES
Column: 250 × 4.6 Supelco C18

Mobile phase: MeCN:buffer 45:55 (Buffer was 44 mM KH_2PO_4 containing 1.5 mL/L triethylamine, adjusted to pH 2.5 with phosphoric acid.)
Flow rate: 1.5
Injection volume: 65
Detector: UV 240

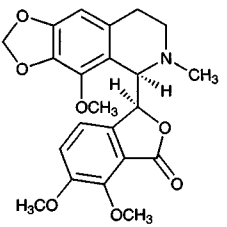
CHROMATOGRAM
Retention time: 9.73
Internal standard: 1-(3-(dimethylamino)propyl)-1-(p-chlorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile (LU 10-202) (Lundbeck, Copenhagen) (8.33)

OTHER SUBSTANCES
Extracted: metabolites, citalopram, amitriptyline
Simultaneous: chlorprothixene, clomipramine, clozapine, flupenthixol, haloperidol, levomepromazine, perphenazine, zuclopenthixol
Noninterfering: benzodiazepines
Interfering: desmethyllevomepromazine

KEY WORDS
serum

REFERENCE
Olesen,O.V.; Linnet,K. Simplified high-performance liquid chromatographic method for the determination of citalopram and desmethylcitalopram in serum without interference from commonly used psychotropic drugs and their metabolites, *J.Chromatogr.B*, **1996**, *675*, 83–88.

Noscapine



Molecular formula: $C_{22}H_{23}NO_7$
Molecular weight: 413.43
CAS Registry No.: 128-62-1, 6035-40-1 (dl-form), 912-60-7 (HCl)
Merck Index: 6815

SAMPLE
Matrix: blood
Sample preparation: Condition a C18 SPE cartridge with 2 mL MeOH, 2 mL water, and 1 mL 50 mM pH 7.4 sodium phosphate buffer at 1.5 mL/min. Centrifuge rapidly thawed plasma samples. Combine 1 mL plasma with 40 µL 903 ng/mL papaverine free base and 2 mL 50 mM pH 7.4 sodium phosphate buffer, vortex. Add 2.75 mL diluted plasma at a rate of 0.36 ml/min to the SPE cartridge, wash with 1 mL 50 mM pH 7.4 sodium phosphate buffer and 2 mL water. Dry by passing 5 mL of air through the SPE cartridge at 5 mL/min. Elute with 1.5 mL MeCN, evaporate the eluate to dryness under nitrogen at 30°. Reconstitute with 200 µL MeCN, inject a 50 µL aliquot.

HPLC VARIABLES
Column: 125 × 4.0 5 µm Nucleosil 120-5-C18
Mobile phase: MeCN:25 mM pH 4.5 sodium phosphate buffer 40:60
Column temperature: 25
Flow rate: 1.0
Injection volume: 50
Detector: UV 211

CHROMATOGRAM
Retention time: 4.99
Internal standard: papaverine (3.98)
Limit of detection: 0.9 ng/mL

Limit of quantitation: 7.2 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Chollet,D.F.; Ruols,C.; Arnera,V. Determination of noscapine in human plasma using solid-phase extraction and high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *701*, 81–85.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 213.4

CHROMATOGRAM
Retention time: 12.827

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.2

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquin-

amide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

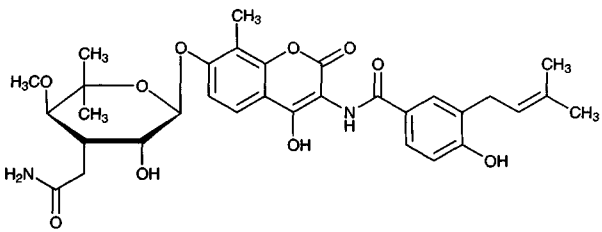
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, dil-

tiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Novobiocin



Molecular formula: $C_{31}H_{36}N_2O_{11}$
Molecular weight: 612.63
CAS Registry No.: 303-81-1, 1476-53-5 (Na salt), 4309-70-0 (Ca salt)
Merck Index: 6818

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL buffer + 4.5 mL MTBE:isopropanol 97.5:2.5, shake at 70 strokes/min for 5 min, centrifuge at 10-15° at 1100 g for 10 min, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 20-25°, reconstitute the residue in 0.5 mL mobile phase, vortex, sonicate for 5 min, inject a 100 µL aliquot. (Buffer was 400 mL 1 M KH_2PO_4, 400 mL K_2HPO_4 solution, and 250 g KCl, adjust pH to 6.5.)

HPLC VARIABLES

Column: 100 × 8 5 µm Nova-Pak C18 Radial-Pak
Mobile phase: MeOH:2-methoxyethanol:buffer 80:5:15 (Buffer was 4.33 g sodium lauryl sulfate and 2 mL 1 M phosphoric acid in 150 mL water, pH 2.8.)
Flow rate: 1.8

Injection volume: 100
Detector: UV 330

CHROMATOGRAM
Retention time: 3.0
Internal standard: novobiocin

OTHER SUBSTANCES
Extracted: coumermycin A1

KEY WORDS
dog; plasma; novobiocin is IS

REFERENCE
Strojny,N.; Conzentino,P.; de Silva,J.A. Determination of coumermycin A1 in plasma by reversed-phase high-performance liquid chromatographic analysis, *J.Chromatogr.*, **1985**, *342*, 145–158.

SAMPLE
Matrix: blood
Sample preparation: 0.5-1 mL Plasma + 10 μL 5 mM prednisone in MeOH + 5 mL MeOH, vortex for 10 s, centrifuge at 2000 g for 10 min, inject a 15 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: CN Guard-Pak
Column: 150 × 4.6 5 μm APEX octyl EC C8 (Jones Chromatography)
Mobile phase: Gradient. MeOH:buffer from 55:45 to 20:80 over 20 min. (Buffer was water acidified to pH 3.0 with trifluoroacetic acid.)
Flow rate: 1.5
Injection volume: 15
Detector: UV 254

CHROMATOGRAM
Retention time: 18.1
Internal standard: prednisone (5.5)
Limit of detection: 5 μM

KEY WORDS
plasma

REFERENCE
Chen,T.-L.; Kennedy,M.J.; Dunlap,V.M.; Colvin,O.M. Determination of plasma novobiocin levels by a reversed-phase high-performance liquid chromatographic assay, *J.Chromatogr.B*, **1994**, *652*, 109–113.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 5 μL 4 mM mitomycin C in MeCN + 400 μL MeCN, vortex, centrifuge at 12000 g for 2 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 15 × 3.2 NewGuard RP18
Column: 220 × 4.6 5 μm Spheri-5 RP18
Mobile phase: MeCN:10 mM phosphoric acid 80:20
Flow rate: 0.8
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 4.53
Internal standard: mitomycin C (6.66)
Limit of quantitation: 1 μg/mL

KEY WORDS
serum

REFERENCE
Zuhowski,E.G.; Gutheil,J.C.; Egorin,M.J. Rapid and sensitive high-performance liquid chromatographic assay for novobiocin in human serum, *J.Chromatogr.B*, **1994**, *655*, 147–152.

SAMPLE
Matrix: blood, milk, tissue
Sample preparation: Blend 10 g tissue with 30 mL 200 mM $(NH_4)H_2PO_4$. Dilute each 1 mL of milk or serum with 3 mL 200 mM $(NH_4)H_2PO_4$. Add 10 mL Tissue homogenate, diluted milk, or diluted serum to 10 (muscle, milk, serum) or 20 (liver, kidney) mL MeOH, swirl vigorously, let stand for 5 min, filter (paper), refilter if not clear, to each 3 mL of filtrate from liver or kidney add 1 mL water, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: Supelcosil LC-18-DB
Column: 150 × 4.6 Supelcosil LC-18-DB
Mobile phase: Gradient. MeCN:MeOH:10 mM phosphoric acid 0:50:50 for 1 min, to 80:0:20 over 19 min, return to initial conditions. (At the end of each day flush the column with the final mobile phase, store in this mobile phase.)
Flow rate: 1 (at end of run set flow rate to 2 mL/min for 5 min then 1 mL/min for 5 min)
Injection volume: 200
Detector: UV 340

CHROMATOGRAM
Retention time: 21
Limit of detection: 10 ppb

KEY WORDS
serum; cow; muscle; liver; kidney

REFERENCE
Moats,W.A.; Leskinen,L. Determination of novobiocin residues in milk, blood, and tissues by liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 776–778.

SAMPLE
Matrix: formulations
Sample preparation: Add 10 mL THF to the peanut oil formulation, make up to 100 mL with mobile phase, sonicate, shake at high speed for 5 min, centrifuge at 2000 g for 5 min, inject a 20 μL aliquot of the clear supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrosorb SI-100
Mobile phase: Butyl chloride:THF:MeOH:acetic acid 88:5:4:3 (Butyl chloride was 50% water saturated prepared by mixing equal volumes of water-saturated butyl chloride and anhydrous butyl chloride.)
Flow rate: 1
Injection volume: 20
Detector: UV 340

CHROMATOGRAM
Retention time: 16
Internal standard: prednisone (UV 254) (10)
Limit of detection: 10 ng

OTHER SUBSTANCES
Simultaneous: impurities, degradation products

KEY WORDS
stability-indicating; normal phase; oils

REFERENCE
Tsuji,K.; Rahn,P.D.; Kane,M.P. High-performance liquid chromatographic method for the determination of novobiocin, *J.Chromatogr.*, **1982**, *235*, 205–214.

SAMPLE
Matrix: milk
Sample preparation: 10 g Milk + 30 mL 200 mM ammonium phosphate, shake. Remove a 10 mL aliquot and add it to 10 mL MeOH, swirl vigorously, swirl occasionally for 5 min, filter (paper), refilter if necessary, inject a 1 mL aliquot.

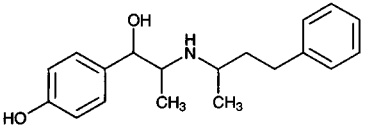
HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-18-DB
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: Gradient. MeOH:5 mM phosphoric acid 50:50 for 1 min, to MeCN:MeOH:5 mM phosphoric acid 80:0:20 over 20 min, maintain at MeCN:MeOH:5 mM phosphoric acid 80:0:20 for 5 min, re-equilibrate at initial conditions for 6 min.
Flow rate: 1 (re-equilibrate at 2 mL/min for 5 min and 1 mL/min for 1 min)
Injection volume: 1000
Detector: UV 340

CHROMATOGRAM
Retention time: 23-25
Limit of detection: <0.05 ppm

KEY WORDS
cow

REFERENCE
Reeves,V.B. Liquid chromatographic procedure for the determination of novobiocin residues in bovine milk: Interlaboratory study, *J.AOAC Int.*, **1995**, *78*, 55–58.

Nylidrin



Molecular formula: $C_{19}H_{25}NO_2$
Molecular weight: 299.41
CAS Registry No.: 447-41-6, 849-55-8 (HCl)
Merck Index: 6830
Lednicer No.: 1 69

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 500 μL buffer, mix briefly, add 50-75 mg resin, mix at 10-25 rpm for 30 min, discard the supernatant, wash twice with 1 mL buffer, add 500 μL 50 mg/mL KOH in MeOH:water 50:50, mix for 30 min, inject a 20 μL aliquot of the eluate. (Buffer was 100 mM citric acid:200 mM Na_2HPO_4 29:71, pH 6.5 (McIlvaine buffer) Wash 20-50 mesh Dowex HCR-S resin twice with water and allow it to equilibrate in buffer).

HPLC VARIABLES
Guard column: 25 × 4.6 5 μm Spherisorb ODS-I
Column: 250 × 4.6 5 μm Spherisorb ODS-I
Mobile phase: MeCN:MeOH:buffer 30:18:52 containing 1.8 mM octanesulfonic acid (Buffer was 30 mM KH_2PO_4 adjusted to pH 3.0 with concentrated orthophosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: E, Gynotek M20, glassy carbon working electrode 950 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 11

Internal standard: nylidrin

OTHER SUBSTANCES
Extracted: isoxsuprine

KEY WORDS
horse; plasma; nylidrin is IS; SPE

REFERENCE
Hashem,A.; Lubczyk,B. Determination of isoxsuprine in equine plasma by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1991**, *563*, 216–223.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

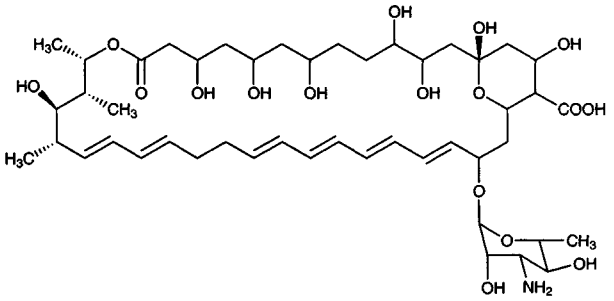
OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasox-

izole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Nystatin



Molecular formula: $C_{47}H_{75}NO_{17}$
Molecular weight: 926.11
CAS Registry No.: 1400-61-9
Merck Index: 6834

SAMPLE
Matrix: CSF
Sample preparation: Condition a BakerBond C18 SPE cartridge with 3 mL MeOH and 3 mL 100 mM pH 9 carbonate buffer. Add 1 mL CSF to the SPE cartridge, wash with 2 mL 100 mM pH 9 carbonate buffer, air dry for 2 min, elute with two 500 μL aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 200 μL MeOH, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:10 mM pH 5 EDTA 35:65
Flow rate: 0.5
Injection volume: 100
Detector: UV 410

CHROMATOGRAM
Retention time: 8.5
Internal standard: nystatin

OTHER SUBSTANCES
Extracted: amphotericin B

KEY WORDS
dog; human; SPE; nystatin is IS

REFERENCE

Liu,H.; Davoudi,H.; Last,T. Determination of Amphotericin B in cerebrospinal fluid by solid-phase extraction and liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1395–1400.

SAMPLE
Matrix: bulk

HPLC VARIABLES
Column: 500 × 1 7-8 μm Zorbax B.P. Sil
Mobile phase: MeOH:DMF:water:acetic acid 72:25:3:0.4

Flow rate: 0.125
Injection volume: 1
Detector: UV 308

CHROMATOGRAM
Retention time: 13

KEY WORDS
microbore; normal phase

REFERENCE
Milhaud,J.; Gareil,P.; Rosset,R. Separation of filipin and nystatin complexes by semi-preparative and microbore high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *358*, 284–287.

SAMPLE
Matrix: bulk
Sample preparation: Make up a 10% solution in DMF then dilute to 20 mg/mL with MeOH: water 66:34, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 500 × 9 10 μm Partisil ODS 2
Mobile phase: MeOH:water:acetic acid 66:33:1
Flow rate: 8
Injection volume: 200
Detector: UV 308

CHROMATOGRAM
Retention time: 22

KEY WORDS
semi-preparative

REFERENCE
Milhaud,J.; Gareil,P.; Rosset,R. Separation of filipin and nystatin complexes by semi-preparative and microbore high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *358*, 284–287.

SAMPLE
Matrix: bulk

HPLC VARIABLES
Column: 300 × 4.5 μBondapak C18
Mobile phase: MeOH:water 70:30
Flow rate: 2
Detector: UV 280

CHROMATOGRAM
Retention time: 10

REFERENCE
Mehta,R.T.; Hopfer,R.L.; Gunner,L.A.; Juliano,R.L.; Lopez-Berestein,G. Formulation, toxicity, and antifungal activity in vitro of liposome-encapsulated nystatin as therapeutic agent for systemic candidiasis, *Antimicrob.Agents Chemother.*, **1987**, *31*, 1897–1900.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve in mobile phase to a concentration of 1 mg/mL.

HPLC VARIABLES
Column: 250 × 4 7 μm LiChrosorb RP-18

Mobile phase: MeOH:water 90:10 + 1% formic acid, pH 3.5
Flow rate: 1
Injection volume: 10
Detector: UV 340

CHROMATOGRAM
Retention time: 6.3

OTHER SUBSTANCES
Simultaneous: impurities

REFERENCE
Sauer,B.; Matusch,R. High-performance liquid chromatographic separations of nystatin and their influence on the antifungal activity, *J.Chromatogr.A*, **1994**, *672*, 247–253.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4 5 μm ODS-Hypersil
Mobile phase: MeOH:DMF:10 mM pH 7.0 Tris in water 56:9.6:34.4
Detector: UV 305

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Egodage,K.L.; Haslam,J.S.; Rajewski,R.A.; Stella,V.J. Correlation and validation to the USP bioassay of a RP-HPLC assay for nystatin (Abstract 3373), *Pharm.Res.*, **1997**, *14*, S587.

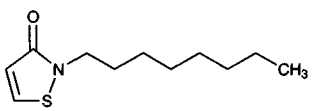
SAMPLE
Matrix: solutions
Sample preparation: Dissolve in DMSO to 10 mg/mL, dilute 1:20 with MeOH.

HPLC VARIABLES
Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:50 mM phosphate buffer (pH 3.5-8.1) 30:70 to 35:65
Flow rate: 0.4-2
Detector: UV 313

OTHER SUBSTANCES
Simultaneous: amphotericin A

REFERENCE
Aszalos,A.; Bax,A.; Burlinson,N.; Roller,P.; McNeal,C. Physico-chemical and microbiological comparison of nystatin, amphotericin A and amphotericin B, and structure of amphotericin A, *J.Antibiot.(Tokyo)*, **1985**, *38*, 1699–1713.

Octhilinone



Molecular formula: $C_{11}H_{19}NOS$
Molecular weight: 213.34
CAS Registry No.: 26530-20-1
Merck Index: 6853

SAMPLE
Matrix: blood, urine